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The process of birth (parturition) has a critical impact on normal human development. Any deviations from the typical parturition process are defined clinically as birth complications, and have been linked to the development of neurological deficits. Two relatively common types of consequences from birth complications are perinatal asphyxia and Caesarean Section delivery (C-section); however, C-section is becoming more common as a matter of choice (elective C-section delivery) rather than as a consequence of some birth complication (Barber et al., 2011; Martin et al., 2012). The two consequences of birth complications differ: perinatal asphyxia involves extended periods of oxygen deprivation during delivery, whereas elective C-section deprives the fetus of the typical conditions associated with a vaginal delivery. Animal research reveals that both perinatal asphyxia and C-section lead to increased expression of dopamine in the mesolimbic dopaminergic pathway. Since this dopaminergic pathway is important for learning, attention, working memory, motivation, movement and mood there is evidence that such increases in dopamine expression result in deficits in these functions. Because the mesolimbic dopaminergic system innervates the hypothalamus, previous research suggested that the complication of perinatal asphyxia results in an increased sensitivity to stress via alterations in the functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Such alterations may be apparent in the development of sensitivity to stressful situations. One test of sensitivity to stressful situations for rodents is to present a resident adult male with an adult male intruder. This social situation can be marked by investigatory and aggressive behaviors on the part of the resident

male. The hypothesis for this thesis is that perinatal asphyxia and C-section delivery of male rats will result in differences in adult investigatory and aggressive social behaviors in this stress test. This study compares the social behavior of 55 day old, postpubertal male rats exposed to asphyxia and C-section at birth, with that of vaginally delivered rats. The social behaviors also were examined in relation to neuroanatomical and neurochemical alterations in dopamine transmission, specifically in the nucleus accumbens.

SOME CONSEQUENCES FOR SOCIAL BEHAVIOR OF PERINATAL ASPHYXIA AND C-SECTION DELIVERY OF FULL TERM MALE RATS

by

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CHAPTER I

INTRODUCTION

Overview of Human Parturition, Asphyxia and Caesarean Section

Parturition is a critical process in mammalian development that is associated with an extensive environmental change, which can subject the fetus to potentially extreme conditions. In some cases, fetuses endure extended periods of oxygen deprivation causing immediate and long-term harm to the central nervous system. This condition of extended oxygen deprivation is clinically defined as perinatal asphyxia. Fetuses at high risk for perinatal asphyxia are surgically delivered through Caesarean section (C-section), which eliminates the experience of normal parturition and labor. Depriving the fetus of normal parturition experiences however, may create another set of negative consequences.

During normal parturition, the human fetus proceeds through the birth canal over the course of several hours. During this time the mother experiences labor through forceful uterine contractions, causing intermittent periods of oxygen deprivation and compressions of the body to the fetus (Lagercrantz & Slotkin, 1986). One crucial response of the fetus to these periods of oxygen deprivation and body compressions is an elevated surge of the catecholamines epinephrine and norepinephrine, which are sometimes described as “stress” hormones. These catecholamines are primarily produced by the adrenal gland. Their production and release mobilizes stored fat and glycogen for cellular nourishment, evacuates fluid from the lungs to facilitate respiration, and shunts blood to the brain and heart (Jones, 1980; Lagercrantz & Slotkin, 1986). Many of the consequences of this catecholamine surge are essential and prepare the fetus

for the extra-uterine environment, therefore an extremely elevated surge and the absence of the surge could present risks during labor.

Newborns suffering the birth condition of perinatal asphyxia, experience extended, rather than intermittent, periods of oxygen deprivation and body compressions. An asphyxiated fetus releases approximately 218nmol/liter of catecholamines in response to the extended periods of oxygen deprivation, which is quite elevated compared to 62nmol/liter of catecholamines released by a normally delivered fetus (Lagercrantz & Bistoletti, 1973). This extremely elevated surge and deprivation of oxygen can physically harm the immature central nervous system. Perinatal asphyxia occurs in 1-9 out of every 1000 live term births in humans (Palsdottir, Daqbjartsson, Throkelsson, & Hardardottir, 2007), and has been linked to the development of cerebral palsy, forms of autism, attention deficit/hyperactivity disorder (ADHD), schizophrenia, and intellectual deficits (Boksa & El-Khodor, 2003; Hagberg, Hagberg, Lewerth, & Lindberg, 1999; Malamud, 1981; McIntosh, Mulkins, & Dean, 1995; Nath, Roy, & Mukherjee, 2012). These long-term negative consequences of perinatal asphyxia however, can be avoided through surgical delivery of the fetus.

Over the past few decades, the number of C-section deliveries has increased, accounting for about 33% of all human births in the United States (Martin et al., 2012). A C-section is a surgical procedure in which the fetus is directly removed from the mother's uterus for delivery (Hamilton, Martin, & Ventura, 2009). Usually a C-section procedure is scheduled ahead of delivery, to ensure the well-being of the mother and fetus when a delivery complication has been identified (i.e. the fetus is incorrectly positioned, or irregularities in heart rate are recorded through electronic fetal monitoring) (NICE, 2007). Although C-sections may remove the risks associated with normal delivery, the experience of birth labor is typically absent and newborns display a significantly lower level of circulating catecholamines compared to normally delivered

infants (Irestedt, Lagercrantz, Hjemdahl, Hägnevik, & Belfrage, 1982). The resulting absence of stress hormones make the newly C-section delivered infant less able to effectively clear its lungs, mobilize fuel for cell nourishment and direct blood flow to its brain and heart. These immediate negative effects have been linked to later development of neuropsychological dysfunctions similar to those arising from perinatal asphyxia (Almqvist, Cnattingius, Lichtenstein, & Lundholm, 2012; Venerosi, Cutuli, Chiarotti, & Calamandrei, 2006; Venerosi, Valanzano, Cirulli, & Calamandrei, 2004).

Although C-sections are only recommended for high risk pregnancies, variability in fetal heart rate interpretation, early induction of labor, multiple gestation, increasing maternal age, and previous C-section deliveries account for unreasonable increases in C-section procedures (Barber et al., 2011; Burns, Geller, & Wholey, 1995; Ecker, Chen, Cohen, Riley, & Lieberman, 2001; VanderWeele, Lantos, & Lauderdale, 2012; Zain, Wright, Parrish, & Diehl, 1998; Zhang et al., 2010). The presence and capabilities of fetal heart rate monitors have increased over the years, however obstetricians fail to reliably assess anomalies in fetal heart rate tracings prior to labor, and advise C-section delivery when anomalies are recorded (Barber et al., 2011; Zain et al., 1998). Furthermore, introduction of synthetic oxytocin (Pitocin) has allowed obstetricians to conveniently schedule deliveries prior to gestational term (VanderWeele et al., 2012). Medical induction of labor through Pitocin however has resulted in higher incidences of labor arrest, forcing delivery by C-section (Barber et al., 2011). Anomalies in fetal heart rates and planned pre-term birth are therefore cases of elective C-section rather than emergency C-section and account for around 60% of first time C-section births (Barber et al., 2011). Elective C-sections and emergency C-sections deprive the fetus from necessary experiential factors, however elective C-sections directly translate to C-sections performed in this study.

Perinatal asphyxia and C-section delivery result in contrasting abnormal exposure to stress hormones. The complication of perinatal asphyxia increases the "oxygen stress" (periods of oxygen deprivation) experienced by the fetus, resulting in an unusually elevated exposure of stress hormones. In contrast, C-sections eliminate the "oxygen stress" associated with normal birth, therefore preventing the typical activation pattern of stress hormones experienced through labor (Irestedt et al., 1982; Lagercrantz & Slotkin, 1986; Ronca, Abel, Ronan, Renner, & Alberts, 2006). The consequences of these contrasting differences in oxygen stress on catecholamine secretions are thought to be responsible for later development of neuropsychological dysfunction. To better understand these consequences to the brain and behavior; researchers have designed animal models mimicking the process of asphyxia and C-section delivery. By understanding the specific developmental consequences and mechanisms of perinatal asphyxia and C-section we can efficiently reroute developmental pathways to a more normal trajectory.

Animal Models of Asphyxia

The lab rat has been the typical animal model of perinatal asphyxia and has helped to identify the neurochemical and behavioral alterations that result from both perinatal asphyxia and C-section delivery. Bjelke et al. (1991) developed the first experimental procedure properly modeling the insult of asphyxia on fetal pups. At the day of gestation (G22), the pregnant dam was put under inhalable anesthetic. One of the two uterine horns was chosen for the asphyxia procedure and then excised from the abdomen of the rat mother and submerged in a heated saline bath for an extended period of time. Excision of the uterine horn disrupts blood gas exchange between the mother and pups, leading to asphyxia. Pups are then delivered through C-section from both uterine horns after the designated length of asphyxia. Therefore, studies investigating the consequences of asphyxia using rats compare differences between three groups: C-section with perinatal asphyxia, C-section without perinatal asphyxia, and normal vaginal delivery.

Wide ranges in periods of asphyxia on fetal rats have been used throughout the literature. Two to six minute periods of asphyxia are recognized as slight asphyxiation, 10 to 16 minute periods are considered to be moderate asphyxiation, and 19 to 22 minute periods are considered to be extreme asphyxiation. Light, moderate, and extreme asphyxia periods, are comparable to what human newborns experienced by the degree structural and brain damage. Fetal pup survival rate for moderate asphyxiation is near 100% while more extreme asphyxiation results in 0-20% survival rate (Y. Chen et al., 1997). Extreme asphyxia deprives the organ systems of the pup for extended periods resulting in high levels of cellular death and therefore pup death. Furthermore, pups from extremely asphyxiated groups are considered to be a biased sample because only very healthy pups survived for investigation. This thesis study investigated the effects of moderate asphyxia (15 min) as it displays effects similar to extreme asphyxia without compromising the survival rate of pups (Boksa & El-Khodor, 2003).

To better mimic the clinical aspects of perinatal asphyxia and C-section in the laboratory we present a modified version of the original Bjelke et al. (1991) method. This thesis study examined Sprauge-Dawley rats at gestational day 22, which is developmentally equivalent to a human fetus in the 3rd trimester and therefore compares to a preterm birth in humans (Clancy, Finlay, Darlington, & Anand, 2007; Dobbing & Sands, 1979). Past studies have administered asphyxia at a more comparable full-term period (Rice, Vannucci, & Brierley, 1981), however it is important to study the entire developmental trajectory of the organism rather than perturb one developmental phase to understand how the perturbation at one phase manages to have an effect (Michel & Moore, 1995; Michel, 2010). Following spinal transection and abdominal incision, the mother's uterine and ovarian arteries on a single uterine horn were ligated for 15 minutes to impair blood gas exchange (Ronca et al., 2006; Ronca & Alberts, 1995). Therefore, the non-ligated uterine horn served as a C-section delivered control for the asphyxiated pups.

Experimentally controlled asphyxiation through delayed C-section delivery serves as an optimal rat model for the study of human asphyxia as it mimics clinical conditions of asphyxia. Although the surgical procedure of C-section allows reliable asphyxiation of pups, C-section alone has been identified as a separate risk factor for the development of schizophrenia, ADHD, and intellectual deficits (Boksa & El-Khodor, 2003; McIntosh et al., 1995). Of course, such atypical psychological functioning may be the result of the complications that provoked the C-section and not the result of that mode of delivery. Nevertheless, elective C-section delivery is not developmentally equivalent to vaginal delivery and should be investigated as a contributor to the development of atypical psychological functioning.

Neural Consequences of Asphyxia and C-Section Delivery

Although perinatal asphyxia and/or C-section have each been linked to later development of neuropsychological disorders and neurological dysfunction, the specific mechanism of this linkage is unknown. One mechanism that might account for the similarities in neuropsychological dysfunction between perinatal asphyxia and C-section is a difference in "oxygen stress" compared to normal delivery. This difference in early stress activation produces immediate and long-lasting alterations in two brain systems: the mesolimbic dopaminergic pathway and the hypothalamic-pituitary-adrenal (HPA) axis (Boksa, 1997; Geddes et al., 1999; McNeil, Cantor-Graae, & Ismail, 2000; Ungethum et al., 1996; Weinstock, 2001; Welberg & Seckl, 2001). Alterations in the development of both of these neural systems can contribute to various forms of neurological dysfunction. By understanding differences in the mesolimbic dopaminergic pathway, we can better pinpoint abnormal alterations in the brain, and links to differences in the developmental trajectory of the relation between the dopaminergic pathway and social investigatory behaviors.

Role of the Mesolimbic Dopaminergic Pathway

The mesolimbic dopaminergic system (MLDA) originates in the ventral tegmental area (VTA) and sends neurons to components of the limbic system, which contributes to the organization and expression of emotion, movement, motivation, memory and olfaction. The limbic system is mainly composed of the nucleus accumbens, amygdala, hippocampus, caudate putamen, prefrontal cortex (PFC) and anterior cingulate cortex. Projections to the limbic system via the nucleus accumbens are of particular importance because they influence the ventral pallidum, hypothalamus, and parts of the frontal lobes (Kandel, Schwartz, & Jessel, 2000). The nucleus accumbens and caudate putamen are important structures for mechanisms that increase the probability of further expression of dopamine (DA) stimulating behaviors, and if altered could lead to deficits in motivated behavior (Graybiel & Saka, 2004). Changes to the hippocampus can lead to deficits in learning, memory and spatial navigation. Changes in DA transmission in the PFC can lead to changes in executive function, such as planning, problem solving, inhibition and expression of social behavior. Furthermore, alterations to the anterior cingulate cortex can lead to changes in reward anticipation, decision-making, empathy and emotion, all closely associated with functions of the PFC. The globus pallidus, if altered, can affect voluntary movement. Even physiological function controlling activities such as drinking, eating, sleeping and sexual behavior (all mediated by the hypothalamus) could be altered (Kandel et al., 2000).

In general, this dopaminergic pathway plays an essential role in reinforcement mechanisms (learning), certain cognitive functions (attention, working memory), voluntary movement, motivation, and mood (Costa, Lin, Cyr, Gainetdinov, & Caron, 2006). Clinical and animal studies on asphyxia and/or C-section have reported differences in development of the MLDA leading to abnormal differences in behaviors regulated by the limbic system. Thus, alterations in dopaminergic activity will propagate throughout the brain, and potentially lead to

abnormalities in the many DA mediated behaviors and developmental trajectories of dopaminergic pathways.

Researchers have investigated neural DA concentrations within the MLDA, and DA mediated behaviors following both asphyxia and/or C-section delivery to further elucidate neural mechanisms of these birth complications. Post-mortem investigation of DA concentrations suggests both asphyxia and/or C-section can lead to long-term over-activity of DA in the MLDA compared to vaginally delivered controls (Bjelke, Andersson, Ogren, & Bolme, 1991; Boksa & El-Khodor, 2003; Boksa, Zhang, & Besawros, 2002; Boksa & Zhang, 2008; Boksa, 1997; Y. Chen et al., 1997). To gain a better understanding of increased DA expression, researchers examine pre-synaptic and post-synaptic properties of neural tissue, while measuring behavioral differences in DA-mediated behaviors following asphyxia and/or C-section deliveries.

Pre-synaptic research on asphyxia and/or C-section supports the hypothesis of increased DA in the MLDA following the birth conditions of asphyxia and/or C-section delivery. DA concentrations are measured indirectly and from DA metabolites, 3, 4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Both DOPAC and HVA are metabolized from DA by monoamine oxidase (MAO) and Catechol-O-methyl-transferase (COMT). Eighty minutes after delivery, asphyxiated pups display significantly increased levels of DA and DOPAC in the substantia nigra, and increased levels of DOPAC and HVA in the VTA compared to vaginally delivered controls. After 8 days, increased levels of DA subside while significantly increased levels of DOPAC in the substantia nigra and VTA continued until approximately 4 weeks of age (Y. Chen et al., 1997). Pups delivered by C-section however, display similar levels of DA and DA metabolites early in development, compared to vaginally delivered controls (Y. Chen et al., 1997). Since DA and DOPAC levels in the MLDA have been shown to decrease within 8 days of birth, multiple time points between 80 minutes and 8 days could elucidate whether this increase is

due to experience of asphyxia and have an impact on early development. Nonetheless, increased DA levels in the MLDA of the asphyxiated pups could lead to disrupted developmental trajectories of MLDA.

At approximately 28 to 35 days of age, rats are considered to be in a prepubertal juvenile stage of development. Although increased levels of DA and DA metabolites increase in the midbrain after 4 weeks, other areas of the MLDA are affected by asphyxia and/or C-section delivery at birth. Specifically, there is a significant increase in monoamine DA and DOPAC in the PFC and the amygdala of 34-day-old asphyxiated rats compared to vaginally delivered controls (Laplante, Brake, Chehab, & Sullivan, 2012). Increases in monoamine levels of DA and DOPAC suggest increases in DA synthesis and up-regulation of DA. Since the PFC receives dopaminergic projections from the VTA, early increases in DA expression in the VTA could lead to increased levels of expression at 35 days of age in the PFC, supporting the notion of altered developmental trajectories. Furthermore, 35-day-old asphyxiated rats display increased levels of the DA precursor tyrosine hydroxylase in substantia nigra and VTA (Bjelke et al., 1991; Boksa & El-Khodor, 2003), further supporting the notion of the increase in DA synthesis and altered developmental trajectories. Rats delivered by C-section and exposed to asphyxia express increased levels of pre-synaptic DA immediately following birth and continuing through 35 days of age. The process of C-section alone however, does not seem to affect DA concentration until early adulthood.

At approximately 55 days of age, the rat is postpubertal and considered to be in adulthood. Following puberty, rats delivered by C-section alone begin to display altered levels of DA in comparison to asphyxiated and vaginally delivered controls (El-Khodor & Boksa, 2001). Fifty-five-day-old rats delivered by C-section only display significantly decreased levels of DA in the PFC and increased levels in the nucleus accumbens and striatum compared to vaginally

delivered controls, which is consistent with the inhibitory effect DA levels in the PFC have on DA transmission in the nucleus accumbens (Vezina, Blanc, Glowinski, & Tassin, 1991). Rats delivered by C-section and asphyxiated however, display similar DA levels in the PFC, nucleus accumbens, and striatum, in comparison to vaginally delivered controls, suggesting potential recovery of the MLDA. Potential recovery of the MLDA in asphyxiated rats contrasted with altered DA levels in the MLDA of littermates delivered by C-section only, suggests the process of C-section has a specific effect on the DA system of the brain that is different if exposed to asphyxia (El-Khodor & Boksa, 1997).

Additional research on post-synaptic properties supports the notion that C-section results in an increased DA profile compared to asphyxia and normal vaginal delivery. Rats born by C-section display increased D1 receptor ligand binding in the nucleus accumbens at 3 months of age compared to normally delivered controls (Boksa et al., 2002), which could be the result of an altered developmental trajectory (Murrin & Zeng, 1990). However, post-synaptic research is limited. Increases in D1 receptors could either result from receptor up-regulation through a reduction in DA secretion (Giorgio, Pibiri, Loi, & Corda, 1993; Joyce, 1990), or a loss of dopaminergic innervation (LaHoste & Marshall, 1991). Measuring DA precursors such as tyrosine hydroxylase in the nucleus accumbens and identifying dopaminergic innervation at the nucleus accumbens would elucidate the mechanism contributing to increases in D1 receptor ligand binding. Furthermore, studying the reuptake of DA by the pre-synaptic DA transporter and degradation of DA by monoamine oxidase would provide additional information about how DA is affecting post-synaptic receptors.

Behavioral studies on rats following asphyxia and C-section delivery provide behavioral evidence for increased DA levels in the striatum and substantia nigra. The striatum and substantia nigra are part of the MLDA that control locomotor activity of the rat. Therefore, increased DA

transmission in the substantia nigra and striatum should lead to increased locomotion and rearing compared to normally delivered controls (Dreher & Jackson, 1989). The first behavioral tests on rats asphyxiated and/or delivered by C-section at birth suggested that at 35 days of age, asphyxiated rats display decreased frequency of rearing and similar frequency of locomotion compared to C-section only littermates. Interpretation of this study is limited, however, by a lack of a normally delivered control group. Therefore the behavioral evidence may not validate the increased levels of DA in 35-day-old asphyxiated rats.

To further elucidate the action of the DA system and behavior, researchers have intravenously injected the DA agonist apomorphine, which floods the MLDA and leads to increases in DA mediated behavior. Following apomorphine administration, asphyxiated rats display increased locomotion behavior compared to asphyxiated rats unexposed to apomorphine and C-section only delivered littermates administered apomorphine. Asphyxiated rats administered apomorphine however, displayed decreased frequency of rearing compared to C-section only littermates, suggesting imbalanced DA expression and neural mediation of rearing and locomotion. However, rats bred for high-levels of anxiety also display decreased frequency of rearing similar to asphyxiated rats (K. Chen, Holschneider, Wu, Rebrin, & Shih, 2004). Furthermore, high anxiety rats and asphyxiated rats display increased levels of locomotion (Boksa & El-Khodor, 2003; Boksa & Zhang, 2008), suggesting a higher sensitivity to apomorphine administration and anxiety-related behavior in asphyxiated rats. Since rats delivered by C-section only have increased D1 receptor-ligand binding it was hypothesized that C-section only rats would display an increase in locomotion behavior compared to asphyxiated rats. However the unexpected finding of increased locomotion and anxiety-related behavior in asphyxiated rats suggests D1 receptors only partly contribute to DA mediated responses, and other post-synaptic and neuroanatomical DA links must be investigated.

The CA1 and CA3 areas of the hippocampus are part of the MLDA and are alternative mediators of locomotor activity (Kandel et al., 2000). Rats exposed to asphyxia at birth display a loss of hippocampal cells in the CA1 and CA3 areas (Bjelke et al., 1991), serving as an explanation for the increased locomotion and decreased rearing behavior. Overall increases in DA transporter in the nucleus accumbens of rats asphyixated and/or delivered by C-section have also been shown to regulate increases in apomorphine induced locomotion (El-Khodor & Boksa, 2002). The MLDA is a complex network wherein D1 receptors, DA transporters, and hippocampal areas are associated with DA mediated behaviors. Increases in D1 receptors of rats exposed to only C-section cannot account for differences in DA mediated behaviors. Observing behaviors following asphyxia and/or C-section may suggest areas for further neural investigation.

Neurochemical and behavioral differences between rats delivered by asphyxia and/or C-sectionmay result from early over-activity of the MLDA, altering the developmental trajectory of the system. Immediately following asphyxia and/or C-section, only rats exposed to asphyxia display increases in DA, DA precursors and DA metabolites (Y. Chen et al., 1997; Laplante et al., 2012). In early adulthood however, rats exposed to only C-section display differences in D1 receptor ligand binding compared to asphyxiated rats (Boksa et al., 2002). Early over-activity in the DA system has been associated with abnormal activation of reinforcement mechanisms, cognition, attention and voluntary movement, and may be related to schizophrenia, ADHD, and intellectual deficits. Schizophrenic clients with hallucinations and delusions display disinhibited release of DA in the striatum (Kandel et al., 2000; Kienast & Heinz, 2006). Increased monoamine DA levels in the prefrontal cortex (Laplante et al., 2012) are associated with attention deficit and impulsivity, both common aspects of ADHD (Graybiel & Saka, 2004).

Thus early increased DA in the MLDA following C-section delivery and/or asphyxia is linked to neurological dysfunction and can contribute to altered developmental trajectories of DA

later in life. Therefore, young adult rats delivered by C-section only should display increases in DA and DA mediated behavior not previously detected in 35-day-old rats. In contrast, asphyxiated rats delivered by C-section should have similar DA levels in comparison to vaginally delivered controls. Asphyxiated and vaginally delivered rats however should display differences in DA mediated behaviors that are correlated with social stress response behaviors and anxiety related behaviors. To better understand these behavioral differences, it is necessary to understand the role of the HPA axis in mediating behavior.

The Role of the HPA Axis

The HPA axis is a complex feedback system between the hypothalamus, pituitary gland and adrenal gland, and is influenced by the nucleus accumbens via the hypothalamus (Kandel et al., 2000). Therefore increased expression of DA can influence the hypothalamus and lead to developmental alterations in the HPA axis. The hypothalamus however also sends and receives signals from the adrenal gland. Thus, atypically extreme or limited levels of adrenal stimulation similar to those associated with both asphyxia and/or C-section delivery can alter the developmental trajectory of the HPA axis (Weinstock, 2001), thus altering basal stress profiles and responses to stress.

The HPA axis signals the release of the hormone corticosterone (CORT) (in rats, cortisol is released in humans) during birth, which is typically secreted for the typical flight-or-fight response. For the system to release CORT in response to a "stressful" situation, a series of cascading steps must occur. During the stressful situation, the limbic system simulates the hypothalamus to release corticotropin-releasing hormone (CRH) along with vasopressin from parvocellular neurons in the paraventricular nucleus. The release of CRH activates the anterior pituitary to release adrenocorticotropin (ACTH), which is potentiated by the presence of vasopressin. ACTH then travels through the blood and attaches to receptors on the adrenal gland,

which releases CORT. CORT influences the activity of the heart, lungs, and capillary dilation. Feedback from these alterations and the influence of CORT on the brain results in the organism displaying a particular set of behavioral responses signifying exposure to a "stressful" situation. CORT also acts as a negative feedback mechanism for its own release, via influences on the hippocampus and hypothalamus. The pattern of feedback affects the adjustment to a stress-inducing situation and marks an individual difference in coping ability. CRH however is not just released by the hypothalamus. Limbic structures in the MLDA such as the nucleus accumbens and amygdala also contain CRH, making alterations in the functioning of the HPA axis important for understanding the consequences of asphyxia and/or C-section.

Too little research has examined the effects asphyxia and/or C-section has on the HPA axis. Two studies have investigated the effects of asphyxia and/or C-section measures of plasma CORT levels and affinities of hypothalamic corticosteroid receptors. Since the release of CORT by the adrenal cortex has a negative feedback effect on hypothalamic functioning, abnormal responses of the hypothalamus from the presence of CORT would indicate that the HPA axis was affected by the experience of asphyxia or C-section delivery. Furthermore, behavioral studies investigating stress responses and anxiety measures following stressful situations can be particularly revealing of the extent to which the HPA axis is affected.

Differences in HPA axis activation between rats asphyxiated and/or delivered by C-section seems to differentially alter developmental profiles of CORT (El-Khodor & Boksa, 1997). One hour after delivery, rats asphyxiated and/or delivered by C-section show significantly decreased levels of CORT compared to vaginally delivered controls. Seven days after birth, rats delivered by C-section only continue to display decreased levels of CORT compared to both asphyxiated and vaginally delivered controls. This decreased level however, significantly increases at 14 days of age in rats born by C-section only in comparison to both asphyxiated and

vaginally delivered rats. Differences in CORT levels subside after 35 days of age across birth condition (El-Khodor & Boksa, 1997), however alterations in early developmental profiles of CORT suggest alterations in the development of the HPA axis.

Mineralocorticoid receptors and glucocorticoid receptors are located in the hypothalamus and hippocampus, and are necessary for CORT regulation, therefore any alterations in these receptors would indicate alterations in HPA axis function and regulation. After puberty and early adulthood, rats asphyxiated and/or delivered by C-section display decreased ligand binding in mineral corticoid receptors, which are activated by low basal levels of CORT (Boksa, Krishnamurthy, & Sharma, 1996). In contrast, glucocorticoid receptors are not significantly different between rats asphyxiated and/or delivered by C-section compared to vaginally delivered controls. Different affinities of glucocorticoid receptors might indicate differences in responses to high levels of stress, however receptor affinities might not directly indicate differences in stress response.

Measurements of CORT secretion following stress-inducing tasks may be more reliable measures in understanding how rats respond to stressful situations and differences in HPA axis response. 3-month-old rats asphyxiated and/or delivered by C-section display significantly reduced CORT levels and similar ACTH levels following 20 minutes of abdominal restraint compared to vaginally delivered controls (Boksa et al., 1996). Decreases of CORT in response to restraint could be a consequence of decreased affinities of mineral corticoid receptors, however similar ACTH levels following restraint suggests differences are more likely due to differences in basal morning CORT levels (Boksa et al., 1996). Nonetheless, alterations in basal morning CORT levels suggest alterations in circadian rhythms indicating differences in HPA axis function.

Since restraint stress stimulates the HPA axis for an extended period of time, stress-inducing tasks such as repeated tail pinch might further elucidate HPA axis functioning. For example, rats asphyxiated and/or delivered by C-section expressed significantly decreased levels of DA transporter in the nucleus accumbens following repeated tail pinch (El-Khodor & Boksa, 2002). This suggests the MLDA systems of asphyxiated and/or C-section rats are more sensitive to repeated stress. Following a mild stressor of a single tail pinch however, C-section with asphyxia rats display decreased DA release in the PFC, whereas C-section only rats must receive repeated tail pinches before there is the same effect of decreased DA release in the PFC (Brake, Sullivan, & Gratton, 2000). This similarity in DA expression following differences in experience suggests rats delivered by C-section and asphyxiated are more sensitive to stress compared to rats delivered by C-section only. Overall differences in developmental CORT profiles, reduced hypothalamic receptor affinities and decreased CORT levels in response to stress are all indicators of HPA axis deficits following asphyxia and/or C-section in rats. Therefore, rats asphyxiated and delivered by C-section should be more sensitive to stressful situations compared to C-section only littermates.

Studies of humans have identified that dysregulation of the HPA axis, recognized as increases or decreases in normal hypothalamic, pituitary and adrenal interactions, may result in stress-mediated abnormalities of neural systems responsible for emotion and cognition (Wilkonson & Goodyer, 2011). Moreover, a growing body of evidence reveals the role of HPA axis dysregulation in schizophrenic symptoms (Harris et al., 2012). Specifically, some clients with schizophrenia symptoms display a blunted cortisol response to psychosocial stress (Brenner et al., 2009). This blunted response also indicates a dysfunction in the sympathetic nervous system. Research also indicates that HPA axis dysfunction in schizophrenia may also be linked to differences in glucose and lactate sensitivity between the central and peripheral nervous systems.

These differences in turn can lead to disruptions in insulin signaling, which may cause decreased synaptic activity in the brain (Harris et al., 2012), therefore leading to neurological deficits.

In summary, perinatal asphyxia and/or C-section may affect the development of dopaminergic pathways and HPA axis function. Dopaminergic alterations in the PFC have been seen in both schizophrenia clients and individuals affected by perinatal asphyxia and/or C-section delivery (Boksa & El-Khodor, 2003; Kandel et al., 2000). Perinatal asphyxia and/or C-section have also been shown to alter dopaminergic pathways in the basal ganglia, which contributes to the ADHD symptom known as impulsivity. The HPA axis also plays a role in neural dysfunction following asphyxia and/or C-section, as unusual catecholamine secretion is a hallmark of the two birth complications in opposite levels. Asphyxiated infants display extremely elevated catecholamine production while C-section infants display an inadequate level of catecholamine production, as compared to a typical birth stress response (Lagercrantz & Bistoletti, 1973). Therefore, differences in catecholamine production during birth will alter the developmental trajectory of the MLDA and HPA axis, therefore affecting further development and social investigative behaviors compared to normally delivered controls.

Investigative and Social Behaviors of Rats Following Asphyxia and C-Section Delivery

Behavioral studies of investigative and social behavior provide further evidence for an over-active MLDA system and dysfunctional HPA axis. Rather than measure specific brain mechanisms and areas, behavioral tests can measure broader components and serve as evidence for new areas of neurobiological investigation. Three methods of examining investigative and social behavior are novelty seeking paradigms, elevated plus mazes and resident/intruder paradigms.

Novelty seeking tests are chosen to investigate differences in exploration and "curiosity" in a novel environment. When 35-day-old C-section only rats are exposed a novel stimulus in this

experiment, they show a lower level of activity and similar levels of investigation in comparison to asphyxiated rats delivered by C-section and vaginally delivered controls (Venerosi et al., 2004). Fifty-five-day-old adult rats asphyxiated and delivered by C-section however investigate novel objects less frequently and display shorter bouts of investigation when compared to C-section only littermates (Morgan, Kleven, Tulbert, Olson, & Ronca, 2012). This suggests that the older asphyxiated rats may be less curious when placed in a novel environment or are at least less curious about novel objects.

In general, 35-day-old rats display high levels of exploration and "curiosity," however rats asphyxiated and/or delivered by C-section do not display differences in exploration and curiosity until early adulthood. Comparing experimental design of these studies reveals that the novelty seeking tests were different for each age group. Novelty seeking tests run on the 55-day-old age group exposed rats to an inescapable novelty, leaving no familiarized place to retreat (Morgan et al., 2012). In contrast, tests run on the 35-day-old age group were in familiarized environments (Venerosi et al., 2004). The inescapable novelty presented to 55-day-old rats could have added another stress component (Misslin, Herzog, Koch, & Ropartz, 1982) that was not present in the 35-day-old age group. Additional research must be done to determine whether asphyxiated rats are investigating the object less frequently compared to vaginally delivered controls and, if so, how this difference develops. Researchers should analyze dopamine receptors in the olfactory bulbs to determine whether the over-active dopamine system is linked via an influence of olfactory sensitivity. Furthermore, different periods of asphyxia such as slight, moderate, and extreme should be administered and novelty tests should be performed in adulthood to determine if the length of asphyxia is leading to reduced investigation.

Elevated plus mazes are historically used to study anxiety related behavior in rats. Rats that remain "frozen" in the middle of the plus maze or retreat to the closed arms are considered to

be displaying increased "anxiety," whereas those with decreased levels of anxiety will explore and investigate the open arms of the plus maze. When placed in an elevated plus maze, aging, asphyxiated rats (24-month-old) demonstrated a higher percentage of entries into the open arms and increased time spent in the open arms in comparison to C-section delivered controls indicating reduced anxiety-related behavior (Hoeger et al., 2000; Weitzdoerfer, Pollack, & Lubec, 2004). Although this finding suggests decreased anxiety in the asphyxiated rats, the novelty seeking tasks suggest increased anxiety. This contradiction in the research may be a consequence of developmental age differences across the studies as well as differences in assessment circumstances. Thus, it is necessary to perform elevated plus maze studies at multiple developmental time points (e.g. from weaning to 55 days) and compare this to novelty investigation studies to better identify the character of this apparent contradiction in results.

Observations of social interactions are important for the investigation of differences in aggressive and defensive behaviors of rats. Most studies use a resident/intruder test for studying such behaviors (Crawley et al., 2007). In this test, unfamiliar rats interact with one another to establish dominance (Albert, Wlash, Gorzalka, Mendelson, & Zalys, 1986). Although dominance is often mistakenly considered to be a trait of an individual, it is actually a dynamic state that is dependent on the experimental condition (C-section only, C-section with asphyxia, or vaginally delivered) and situational context (Michel & Moore, 1995). Since the behaviors associated with achieving dominance are different from non-aggressive social behaviors, dominance must be achieved before non-aggressive social behaviors can emerge in the interaction (Fernandez-Espejo & Mir, 1990). Social behavior tests performed on 35-day-old rats indicated that C-section rats have a higher frequency and longer duration of investigative behaviors, compared to C-section with asphyxia rats and vaginally delivered controls (Venerosi et al., 2004). These results are partially concordant with novel object data collected on 55-day-old rats (Morgan et al., 2012).

This suggests that more social behavior tests might elucidate what type of investigative and other social behaviors are being expressed.

Previous longitudinal studies on behavior for rats asphyxiated and/or delivered by C-section continue to support these developmental differences. Postpubertal 55-day-old asphyxiated rats display increased locomotion behavior and DA release following amphetamine administration whereas prepubertal rats did not (Juarez, Silva-Gomez, Peralta, & Flores, 2003). Future studies should continue to use longitudinal samples to determine whether or not age is a contributing factor to the behavioral effects of asphyxia and/or C-section delivery. The current thesis study uses 55-day-old rats, since this age represents post-pubertal young adult status for the rat, and lacks investigation into social behavior.

Little is known about long-term alterations in social behavior following asphyxia and/or C-section. Studies investigating social behavior on vaginally delivered rats use resident/intruder paradigms to focus on behavior patterns during aggressive interactions (Albert et al., 1986). Male rats in the presence of a strange adult male conspecific usually manifest these patterns of aggressive interactions. Attack behaviors typically emerge as a way to establish dominance (Moyer, 1971). These are aggressive offensive behaviors, and are mostly performed by the "resident" rat (the first rat placed in the arena and initially familiarized with it), who rarely if ever displays defensive behaviors (Fernandez-Espejo & Mir, 1990). Affiliative behaviors such as allogrooming are also recognizable indicators of dominance with the dominant rat being groomed more often. Such allogrooming maintains the social dynamic bonds in the relationship and can serve as a replacement to self-grooming (Spruijt, van Hoof, & Gispen, 1992).

Once dominance has been established, non-aggressive behaviors such as investigatory behaviors (social and non-social) are more prevalent (Fernandez-Espejo & Mir, 1990). The intruder rat, in response to an offensive aggressive attack of the resident rat, displays mostly

aggressive-defensive behaviors. Aggressive-defensive behaviors, or submissive behaviors such as withdrawal and freezing are seen following an aggressive action such as a bite to the nape of the defending rat, or following a dominant behavior such as a rear mount. Aggressive-defensive behaviors are not the only responses to a dominant or offensive aggressing rat. Self-grooming, a type of stress coping behavior, has been identified as a de-arousal technique because it occurs after the termination of, or habituation to, a stressful situation or interaction (Spruijt et al., 1992). Thus, many different social behaviors can be expressed when two male conspecific rats interact. It is important to investigate all of these behaviors in order to elucidate the dynamic social status of each individual in the interactions. This study describes the social behaviors of the interactions of the two rats in investigate this dynamic social state. The study was not designed to describe the sequences of behaviors during the interaction.

In sum, the current study investigates the effect of perinatal asphyxia and/or C-section delivery on the social behavioral interactions of postpubertal (55-day-old) adult male rats. Since increased DA expression in the caudate putamen increases the likelihood for behaviors to continually repeat and make future behaviors more probable, rats with increased DA in the mesolimbic DA pathway should display an increased frequency of investigative behaviors (Graybiel & Saka, 2004). This over-activity has been specifically identified in C-section only rats with increases in D1 receptor ligand binding, and identified in C-section with asphyxia rats as increases in monoamine DA levels (Boksa & El-Khodor, 2003). Therefore rats exposed to asphyxia and/or C-section should display increased frequency of investigative behaviors due to an increase in DA in the caudate putamen. However, rats exposed to asphyxia and C-section delivery should display increased anxiety related behavior, which should lead to decreased social interaction as compared to only C-section delivered and vaginally delivered controls. Furthermore, depending on the severity of the asphyxia and/or C-section experience, there may

be a dysregulation of the HPA axis (Boksa & El-Khodor, 2003) wherein C-sectioned rats displayed blunted CORT responses to stress. Thus, asphyxiated and/or C-section delivered rats should display decreased stress-mediated behaviors compared to vaginally delivered controls. Although studies have suggested differences in prepubertal (35-day-old) and postpubertal (55-day-old) behavior, which are reflected in neurochemical differences in the brain (Juarez et al., 2003), the present study focuses on the long-term consequences on social behavior in the young adult male rat.

Goals and Hypotheses

The goal of the study is to examine the social behavioral and neurochemical differences among rats born via three birth processes; C-section with asphyxia (C-APX), C-section with no asphyxia (C-NON), and typical vaginal delivery (VG). C-APX rats have been reported to display increased anxiety related behavior in comparison to both C-NON and VG rats (Venerosi et al., 2006). Morgan et al. (2012) exposed rats to a novel object in a non-escapable environment and found that C-APX rats display decreased frequency and duration of investigation of the novel object in comparison to C-NON rats. Since social behavior tests also present the rat with a non-escapable environment, It would not be surprising if the findings of Morgan et al. (2012) novel object investigation findings would generalize to social investigation behavior of a conspecific. Therefore, it is hypothesized that C-APX rats will display decreased frequency and duration of social investigative behavior compared to C-NON and VG rats. Decreased social interaction of the C-APX resident with the VG intruder will therefore lead to decreased frequency and duration of dominant behavior, decreased frequency and duration of aggressive-offensive behavior and increased frequency and duration of non-social investigative behavior displayed by the C-APX resident (Fernandez-Espejo & Mir, 1990; Koolhaas, Schuurman, & Wiepkema, 1980).

C-NON rats exhibit increased D1 receptor ligand binding in the nucleus accumbens compared to C-APX and VG rats (Boksa & El-Khodor, 2003), increased locomotion response to amphetamine compared to VG rats (Boksa & Zhang, 2008; El-Khodor & Boksa, 1998), and increased frequency and duration of social investigative behavior compared to C-APX and VG rats (Venerosi et al., 2006). Given previous reports of increased DA activity in the MLDA pathway, it is hypothesized that C-NON rats will display increased frequency and duration of social investigative behavior compared to C-APX and VG rats. Increased interaction of the C-NON resident rat with the VG intruder rat will therefore lead to increased frequency and duration of dominant behavior, and increased frequency and duration of aggressive-offensive behavior compared to C-APX and VG rats (Fernandez-Espejo & Mir, 1990; Koolhaas et al., 1980).

Furthermore, it is hypothesized that the presence of an intruder presents a stressful situation for the resident rat. Studies by Boksa at al. (1996) show that asphyxiated and/or C-section rats display decreased levels of CORT compared to vaginally delivered controls following stressful restraint. Therefore it is hypothesized that C-NON and C-APX rats will display decreased frequency and duration of self-grooming behavior compared to VG controls, with VG controls increasing self-grooming behavior in the presence of an intruder compared to no intruder (Spruijt et al., 1992).

Neuroanatomical and neurochemical studies have revealed some of the long-term effects of asphyxia and/or C-section on the MLDA. Since previous studies suggest over-active DA in the MLDA, it is hypothesized that both C-APX and C-NON rats will display increased levels of TH in the nucleus accumbens. Stress studies performed by repeated tail pinches on adult rats showed decreased levels of DA transporter in the nucleus accumbens (El-Khodor & Boksa, 2002). Social behavior tests are recognized as a stressful interaction for the resident rat; therefore,

it is hypothesized that both C-APX and C-NON rats will display decreased levels of DA transporter in the nucleus accumbens (c.f. El-Khodor & Boksa, 2002).

To examine these hypotheses, a resident/intruder paradigm was used to measure the social behavior of male rats 55 days following birth. Also, immunohistochemistry assays were used to test the neurochemical differences in the nucleus accumbens and caudate putamen of postmortem brain tissue of the same rats at 60 days of age rats following tests of their social behavior.

CHAPTER II

METHODS

Subjects

Full term Sprague Dawley rats were used for all birth conditions that occurred on gestational day 22 (G22, sperm positive = G0). The day of conception was identified by daily examination of vaginal cytology for presence of sperm. Following timed-matings, dams were housed in maternity cages in groups of three under standard colony conditions (12:12 light/dark cycle [0600:1800]; 21ºC at 30-50% humidity)

Twenty-seven rats were either delivered by C-section or vaginally. Prior to delivery on G22, C-section delivered dams were administered spinal anesthesia, an abdominal incision was made and the uterus was externalized into a heated (37.5ºC) buffered saline bath. Once the uterus was externalized, one uterine horn was chosen for the asphyxia condition (C-APX) and the other horn was chosen for the non-asphyxiated condition (C-NON). The uterine horn chosen for C-APX was then occluded at the ovarian and uterine arteries, and pups were delivered after 15mins of occlusion. Following delivery the pups were administered gentle tapping until breathing became even. C-NON pups were delivered at the same time as C-APX but were given no period of asphyxia. The umbilical cord was then ligated and pups placed in an incubator at 36.5ºC for 1 hour until they were fostered to surrogate dams. Pups born vaginally on G22 served as controls and were cross-fostered to surrogate mothers after delivery. On P21, offspring were weaned and housed with same-gender littermates.

Resident/Intruder Test

Rats were weaned at 21 days of age and a modified resident/intruder test (Crawley et al., 2007; File, 1980) was performed at 55 days. Rats to be used in Resident/Intruder tests were not housed together; no littermates interacted with each other. 24 hours prior to the behavioral test, resident (target) and intruder rats were housed solitarily. On the day of the test, rats were placed in a clear, Plexiglas open-field testing arena for all behavioral measures. Preceding the social behavior test, C-APX, C-NON, and VG "resident" rats were exposed to ambient light, dark for 10 minutes each and a novel object for 5 minutes, with a baseline period of 2 minutes in between each period. After these tests and another baseline period, the rat was exposed to a VG unfamiliar intruder matched for sex, age and weight. Social interactions were recorded with a video camera. Behaviors for both resident and intruder were recorded for frequency, duration and latency using ObserverXT (v10 on Windows 7). The behaviors were categorized as follows (in Table 1)

Immunohistochemistry

At 8 weeks of age (one day after social testing), rats were intracardially perfused with 4% paraformaldehyde under a deep plane of isoflurane anesthesia (5% in 100% O_2) and brains harvested. For sectioning, their brains were frozen with cooled (-40º) isopentane in an Optimal Cutting Temperature (O.C.T.) filled mold for 1min. Brains were sectioned coronally at 60µm using a cryostat (Leica Model #CM 1850 UV). Sections were collected at the prefrontal cortex, anterior of the caudate putamen and the amygdala (0.6mm-1.68mm post bregma). Sections were placed into wells and bathed in an antifreeze solution (25% glycerol, 25% ethylene glycol and 50% PB; -20º) for at least 24 hours prior to DA transporter and TH immunohistochemistry. This process of immunohistochemistry tags and therefore identifies the concentration of DA

transporter or TH within the measured area, in this case, the nucleus accumbens and caudate putamen.

Data Analysis

Examination of the data revealed that neither the frequency nor the duration of behaviors followed a normal distribution. Therefore, distribution free, non-parametric tests such as, the Mann-Whitney U and Wilcoxon Signed Rank tests were used for data analyses (Siegel, 1956). For these two tests, raw data are ranked regardless of condition and rank totals for the two conditions are then compared to determine whether there is a systematic difference between the two conditions. The Mann-Whitney U test is used to compare two independent groups, and is similar to a "two-sample" t-test. The Wilcoxon Signed Rank test is used to compare two dependent groups, and ranks the difference between the "repeated measures" or in this case, the two animals in the same litter (Siegel, 1956).

The current study used Mann-Whitney U tests to compare latency, frequency, duration and frequency change over time of the 4 behavior groupings (social investigatory, non-social investigatory, aggressive defensive behavior and aggressive offensive behavior), between C-APX and VG, and C-NON and VG. Wilcoxon Signed Ranked tests were used to compare latency, frequency, duration and frequency change over time of the 4 behavior groupings (social investigatory, non-social investigatory, aggressive defensive behavior and aggressive offensive behavior), between C-APX and C-NON.

CHAPTER III

RESULTS

Frequency and Duration of Investigative and Aggressive Behaviors

Nonparametric tests yielded no significant differences in frequency and duration of grouped social investigative behavior and grouped non-social investigative behaviors (Table 2). Since differences in social investigative behavior were expected, individual behavioral actions were analyzed further rather than grouped behaviors. Analysis of social investigatory behaviors displayed C-NON rats participated in longer bouts of anogential sniffing ($U = 15$, $p<.05$) when exposed to an intruder in comparison to VG controls (Figure 1). C-APX, however displayed no statistical difference in bouts of anogenital sniffing compared to C-NON and VG controls.

It was hypothesized that increases in social investigative behavior would yield increases in aggressive offensive behavior, however the analysis did not support this expectation. Despite variability in frequency of aggressive attacks, C-APX, C-NON, and VG controls displayed similar frequency and duration of aggressive attacks and dominant behaviors. Analysis of aggressive defensive behaviors displayed that C-APX rats were more defensive ($U = 8.5$, $p<.01$) and they withdrew ($U = 11.5$, $p<.05$) from social investigations and aggressive offensive interactions more often than VG delivered controls (Figure 2 & 3). However, intruder rats paired with C-APX rats displayed more frequent aggressive attacks (Figure 4)

Latency of Investigative Behaviors

Latency of the resident and intruder rat to approach the other represents the starting time for the first social interaction during the 10min observational period. C-APX resident rats displayed shorter latency to approach the intruder rat in comparison to C-NON litter mates ($W = 3$, $p<.05$). VG control intruder rats displayed a longer latency to approach C-APX resident rats compared to C-NON resident rats ($U= 15$, $p<.05$) and VG control residents ($W = 7$, $p<.001$) (Figure 4). Furthermore, C-APX resident rats initiated the first social interaction 72% of the time when presented with a VG intruder. However, when C-NON and VG residents were presented with an intruder, they only initiated the first social interaction 12% and 22% of the time, respectively. Therefore, intruders were more likely to approach C-NON and VG rats compared to C-APX rats, and C-APX rats were more likely to approach intruders compared to C-NON and VG rats, X^2 (2, N=24) = 6.634, $p<.05$ (Figure 5).

Self-Grooming Behavior

Nonparametric tests yielded no significant differences among groups in frequency and duration of Self-grooming behavior (Figure4).

TH and DA Transporter

Nonparametric tests yielded no significant findings among groups in average optical density of DA transporter proteins and TH in the nucleus accumbens shell, nor nucleus accumbens core (Table3).

CHAPTER IV

DISCUSSION

Social Investigative Behaviors

Analyses of social investigative behaviors yielded no significant findings among the three groups when all social investigative behaviors were combined. Although all social investigative behavior was predicted to be increased for C-NON rats and decreased for C-APX rats compared to VG controls, only C-NON rats displayed significant increases in duration of anogenital sniffing. Longer bouts of anogenital sniffing are representative of repetitive social investigative behavior, possibly indicating increased DA release in the caudate putamen, potential increases in investigatory motivation, and/or deficits in odor discrimination.

Repetitive or compulsive behaviors seem to stem from imbalances in the direct (striatonigral) and indirect (striatal pallidal) pathways of the basal ganglia. Normally DA mediated behaviors occur following the stimulation of the direct and indirect pathways, and repetitive behaviors occur following suppression of the indirect pathway (Graybiel & Saka, 2004; Langen, Kas, Staal, van Engeland, & Durston, 2011). The direct pathway first involves stimulation of D1 receptors in the striatum from DA projections of the substantia nigra. The post-synaptic receptors with D1 receptors then stimulate sensorimotor areas of the cortex directly, and stimulate a positive feedback loop through the thalamus. This positive feedback loop will continue to stimulate the sensorimotor cortex resulting in repetitive behaviors, until the stimulation of the indirect pathway prevents the feedback. This indirect pathway begins with stimulation of D2 receptors in the striatum, which signals negative feedback to the substantia nigra through the globus pallidus. Thus, stimulation of the indirect pathway through D2 receptors

inhibits further stimulation of the sensorimotor cortex by inhibiting neurons in the substantia nigra involved with the direct pathway (Graybiel & Saka, 2004; Langen et al., 2011). Therefore, C-NON rats displaying repetitive anogenital sniffing behaviors could have decreased D2 receptors in the striatum of the basal ganglia, preventing inhibition of repetitive behavior. Repetitive behaviors however might also indicate signs of increased motivation derived from changes in other neurobiological systems rather than disturbances in D2 receptors.

States of motivation in relation to curiosity are thought to be a product of "wanting" and "liking" states within the MLDA (Berridge & Robinson, 1998). "Wanting" involves stimulation of the striatum and is associated with motivated approach behavior and learned incentives for rewards (Panksepp, Knutson, & Burgdorf, 2002). "Liking" involves the nucleus accumbens and the release of opioids, stimulating a hedonic or pleasure state. Stimuli that are "wanted" are usually "liked"; therefore, both neural systems are stimulated and form a feedback loop (Berridge & Robinson, 1998). These two systems however can be stimulated in different ways and they can exhibit differential adaptation (sensitivity) to repeated activation. For example, increased activation of the "wanting" system with little activation of the "liking" system is associated with impulsive approach-oriented behavior (Panksepp et al., 2002). Therefore, C-NON rats may be investigating more because of increased activation within the striatum, and may experience very little pleasure. Nevertheless, the action is repeated. If increased motivation is leading to longer bouts of anogenital sniffing, it should also lead to higher frequency and longer bouts of all social and non-social investigative behavior. Since C-NON rats only displayed repetitive behaviors in anogenital sniffing rather than overall investigatory behaviors, it is likely that this change is associated with changes in the olfactory pathways.

Anogenital sniffing in rats stimulates the main olfactory system through odorant detection, and the accessory olfactory system through pheromone detection. Stimulation of the

main olfactory system involves cholinergic, noradrenergic, and serotonergic pathways, which link back to the limbic system. Therefore, any stimulation through odorants can stimulate the potential over-active dopaminergic system of rats asphyxiated and/or delivered by C-section. Furthermore, D1 and D2 receptors within the olfactory bulb influence odor detection thresholds and odor discrimination (Escanilla, Yuhas, Marzan, & Linster, 2009). When D1 receptors are stimulated more than D2 receptors, rats show enhanced performance of odor detection. However when D2 receptors are stimulated more than D1 receptors, rats show deficits in performance of odor detection (Doty & Risser, 1989; Doty et al., 1998). Since C-NON rats are participating in longer bouts of anogenital sniffing than VG delivered controls, C-NON rats may be demonstrating deficits in odor detection or discrimination. Therefore, over-active DA from the MLDA might extend to the olfactory bulbs, leading to either increased stimulation of D2 receptors or inhibition of D1 receptors in the C-NON rats, resulting in increased anogenital sniffing. However, this hypothesis does not discuss potential stimulation of the accessory olfactory system during anogenital sniffing.

Stimulation of the accessory olfactory system during anogenital sniffing involves detection of odors excreted from the rat being sniffed resulting in a chemical message being transported to the sniffing rat via the vomeronasal pathway (Halpern & Martínez-Marcos, 2003). Perinatal research on the vomeronasal pathway indicates that the projections associated with the vomeronasal pathway are not developed enough for differences in catecholamines associated with asphyxiation and C-section to directly affect the vomeronasal pathway (Coppola & Millar, 1994). However, projections of the vomeronasal pathway are directly associated with the MLDA, and therefore alterations in the MLDA may influence development of neural connections made with the vomeronasal pathway, which would influence behavior.

Immediately following stimulation of the vomeronasal pathway through receptor-ligand binding of the excreted odor, a signal is sent directly to the amygdala (Lanuza et al., 2008). Stimulation of the MLDA via the amygdala, therefore implicates the vomeronasal pathway in potential behaviors associated with "wanting" and "liking" motivation. However, research suggests the MLDA seems to play a modulatory (inhibitory) role in the processing of socially acquired pheromones, which might act through D1 receptors (Lanuza et al., 2008). Therefore, differences in D1 receptor expression and/or development could influence duration of anogenital sniffing in C-NON rats. The exact mechanisms involved, however, cannot be postulated for rats asphyxiated and/or delivered by C-section until more studies investigate the modulating effects of DA on social discrimination involving the vomeronasal pathway.

C-NON rats displayed longer bouts of anogenital sniffing however the current study cannot specify the neural mechanism for such behavior. Since rats delivered by C-section are more likely to have over-active dopaminergic systems, it is likely that DA plays a role in such repetitive behavior. This repetitive behavior could stem from decreased levels of D2 receptors associated with the indirect pathway of the basal ganglia, leading to suppressed negative feedback and repetition of anogenital sniffing (Langen et al., 2011). Longer bouts might also be evidence for increased motivation states, wherein increased DA activity leads to increased "wanting" without opioid release, thus the behavior is repeated (Berridge & Robinson, 1998). Decreased D2 receptors and increased motivation however should lead to increased overall investigatory behavior; however, C-NON rats only displayed longer bouts of anogenital sniffing. A more specific mechanism involves increased activation of D2 receptors in the olfactory bulb leading to decreased performance in odor discrimination (Doty & Risser, 1989; Doty et al., 1998). However, anogenital sniffing also involves stimulation of the vomeronasal pathway, which may also be influenced by expression of D1 receptors (Lanuza et al., 2008). Future studies should seek to

replicate the increased anogenital sniffing in C-NON rats, investigate the presence of D2 receptors in the basal ganglia and olfactory bulbs, measure D1 receptors associated with the amygdala and measure opioid release following stimulation of the nucleus accumbens to help identify this mechanism.

Although anogenital sniffing in C-APX rats increases similarly to C-NON rats, durations of anogenital sniffing in C-APX rats were not significantly increased compared to VG delivered controls. Novelty seeking tests suggested that C-APX rats would exhibit lower levels of investigatory behavior (Morgan et al., 2012); therefore novelty-seeking results did not generalize to social behavior studies as anticipated. The raw data, however, suggests that C-APX rats were more similar to C-NON rats, suggesting that the insult of asphyxia might not be very different from the insult of C-section only.

Implications of Stress and Anxiety

Placing an unfamiliar rat into a resident's arena was considered a stressful situation for the resident rat (Koolhaas et al., 1980; Misslin et al., 1982), and differences in response to the intruder across experimental condition (C-APX, C-NON, and VG) were hypothesized as evidence for differences in HPA axis functioning and regulation. Rats asphyxiated and/or delivered by C-section display decreased levels of CORT when exposed to stressful situations (Boksa et al., 1996). Therefore, it was hypothesized that rats delivered by C-section and/or asphyxia would exhibit decreased frequency and duration of self-grooming behavior compared to vaginally delivered controls since higher levels of self-grooming are evidence of stress coping (Spruijt et al., 1992). Rats across the three birth conditions however showed similar levels of self-grooming behavior throughout the 10min interaction period, suggesting no differences in stress response. The latency to approach and aggressive-defensive behaviors, however, suggest decreased functioning of the HPA axis as hypothesized.

At the beginning of each social interaction, the intruder rat is placed in the resident's arena and either the intruder or the resident rat approaches the other. This marks the start of social interaction and establishing dominance (Moyer, 1971). Our data shows that when an unfamiliar vaginally delivered intruder is placed with a C-APX resident, the C-APX resident more quickly approaches the unfamiliar intruder rat than the littermates delivered by C-section and VG delivered controls. Comparison of rats who initiated the first interaction further validated the decreased latency, demonstrating that asphyxiated rats delivered by C-section are more likely to approach the intruder first compared to both C-section only delivered littermates and vaginally delivered controls. This difference in behavior can be explained by either increases in motivational behavior or decreases in stress response behavior.

The over-active dopaminergic system of rats asphyxiated and/or delivered by C-section influences both "wanting" and "liking" pathways associated with motivated behavior. Increased release of DA in the striatum stimulates the "wanting" pathway of motivated behavior (Panksepp et al., 2002), leading to decreased latency to approach behavior. Since only C-APX rats displayed significant increases in approach behavior compared to C-NON and VG controls, C-APX rats might have increased release of DA in the striatum compared to C-NON and VG controls. However, both C-APX and C-NON rats have been shown to display over-active dopaminergic systems; therefore, decreases in stress response behavior might better explain why C-APX rats approach quicker than C-NON and VG rats.

Rats asphyxiated and/or delivered by C-section have significantly reduced CORT levels when presented with a stressful situation (Boksa et al., 1996). When C-APX and C-NON rats endure 20 minutes of abdominal restraint, they display decreased CORT release compared to VG controls. Blunted CORT responses to stress might explain why C-APX rats displayed decreased latency to approach behavior, however both C-APX and C-NON rats should show decreased

latency to approach. Further research on stress response behavior is needed because differences in CORT levels between C-APX and C-NON rats might only partly explain decreased latency to approach.

When C-APX rats are administered a stressful tail pinch, they display decreased DA release in the PFC. In contrast, C-NON rats must receive several repeated tail pinches to display a decrease of DA release in the PFC (El-Khodor & Boksa, 2002). Therefore, C-APX rats might be more sensitive to stressors similar to tail pinches. The combination of reduced CORT levels and differences in DA release in stressful situations might better characterize the mechanism involved in the quicker approach behavior by C-APX rats. However, further research should measure CORT levels before, after, and (somehow) during social interactions. In this study, decreased latency to approach lead to further differences in aggressive behavior throughout the 10min interaction period.

Aggressive-offensive behaviors are usually displayed by resident rats to establish dominance during social interactions with intruders (Moyer, 1971). Furthermore, more frequent investigative behavior usually leads to more frequent aggressive-offensive behavior, and less frequent investigative behavior is associated with less frequent aggressive-offensive behavior (Fernandez-Espejo & Mir, 1990). Since C-APX rats were expected to display decreased frequency and duration of social investigative behavior, they were predicted to engage in less aggressive-offensive interactions compared to VG controls (Fernandez-Espejo & Mir, 1990; Koolhaas et al., 1980). In contrast, C-NON rats were predicted to display increased frequency and duration of social investigative behavior, and would engage in more aggressive-offensive interactions compared to VG controls. Analyses of aggressive-offensive behavior in residents revealed no significant differences among the groups. However, analyses of aggressive-offensive behavior in intruders showed that intruder rats interacting with C-APX residents had a higher

frequency of aggressive-offensive interactions. C-APX residents responded with more frequent aggressive-defensive behaviors compared to C-NON and VG delivered controls. This interaction reveals potential differences in the endocrine system of C-APX compared to C-NON and VG delivered controls.

Individual levels of aggressive behavior during an interaction period are important indicators of behavioral physiological response patterns to differing environmental demands. Those animals that display more aggression and are protective of their territory tend to be proactive and flee when receiving offensive actions rather than passively accept the offensive action. Although increases in aggression could provide evidence for increases in motivation to aggress, increases in aggression are associated with decreased parasympathetic reactivity, low pituitary-adrenocortical reactivity, and increased DA metabolite levels in cerebrospinal fluid (de Boer, van der Vegt, & Koolhaas, 2003; Henry & Stephens, 1977). Rats exhibiting more aggressive-defensive withdrawal behavior tend to also have higher sympathetic reactivity to stress (de Boer et al., 2003), and higher post-stress levels of CORT than control rats exhibiting decreased levels of withdrawal behavior and more frequent freezing (Pletzer, Klimesch, Oberascher-Holzinger, & Kerschbaum, 2007). Therefore, C-APX rats displaying more defensive retreats may be responding to the situation because of differences in HPA axis and MLDA function. However, C-NON and VG rats did not experience frequent aggressive-offensive attacks from intruders, and C-APX rats did experience these attacks. Therefore, C-NON and VG rats may respond similarly if they experienced more frequent attacks. Nonetheless, differences in aggressive behavior are associated with differences in endocrine patterns; future resident-intruder paradigms on perinatal asphyxia and C-section must measure endocrine responses to support these differences in HPA axis function.

Although increased self-grooming suggests increased coping of stress (Spruijt et al., 1992), differences in approach and aggressive behavior were more revealing of stress response than levels of self-grooming. Neither C-APX, C-NON, nor VG controls displayed any differences in levels of self-grooming during the 10 minute social interaction period, raising the question of whether the interaction period is long enough. C-APX rats however displayed quicker latency to approach the intruder compared to C-NON and VG controls. Furthermore, C-APX rats approached intruders first, while C-NON and VG controls allowed intruders to approach them first. This difference in approach behavior seems to have led to increased aggressive-defensive behavior in C-APX residents compared to C-Non and VG controls. Increased aggressive-defensive behavior in response to aggressive-offensive attacks suggests higher levels of stress during social interaction than normal (de Boer et al., 2003). Therefore, C-APX rats appeared to display increased stress responses during social interaction tests while C-NON and VG did not. However, C-NON and VG rats did no experience very many aggressive-offensive attacks. Future research must measure neuroendocrine responses to social intruders, and expose residents to interaction periods lasting longer than 10 minutes, to better understand the aggressive behavior and reveal more about stress responses in rats asphyxiated and/or delivered by C-section.

TH and DA Transporter Levels

C-APX and C-NON rats were predicted to express increased concentrations of TH and decreased concentrations of DA transporter in the nucleus accumbens core and shell, supporting over-activity of DA in the MLDA. There were no significant findings in TH concentrations nor DA transporter concentrations in the nucleus accumbens between C-APX, C-NON and VG rats. Following the addition of four animals not used for the social behavioral analyses, significant differences appeared in TH between C-NON and VG delivered controls. However caution must

be taken in the interpretation of such results, and all findings should be replicated before any future investigations can be based of these findings.

Previous studies measured DA transporter after 35-days-age or following a stress study, such as repeated tail pinches. Because resident-intruder paradigms involve social stress, it was hypothesized that C-APX and C-NON rats would display decreased DA transporter levels in the nucleus accumbens compared to VG controls based on previous research measuring DA transporter levels following stress from repeated tail pinches (El-Khodor & Boksa, 2002). However, there were no significant findings when using C-APX, C-NON and VG animals that were part of the social behavioral test and analyses. Further analyses with the addition of four animals (2 C-APX and 2 C-NON) yielded significant findings in the nucleus accumbens shell between C-NON and VG delivered controls, with C-NON animals expressing higher levels of DA transporter compared to VG delivered controls in the nucleus accumbens shell. These four added animals were not included in the social behavioral because they were either exposed to a littermate, C-NON, or C-APX rat rather than a VG delivered control. Therefore, these added animals most likely experienced a different type of social situation compared to animals included in the original analyses. Furthermore, not finding significant differences in the original analyses suggests that our findings in the larger group are not robust and should be taken with caution. Nonetheless, no significant findings between C-APX and VG animals support previous research indicating potential recovery of the MLDA in post-pubertal asphyxiated rats.

Post-pubertal rats asphyxiated and delivered by C-section display similar DA levels in the PFC, nucleus accumbens, and striatum (caudate putamen) compared to VG controls, suggesting potential recovery of the MLDA system (El-Khodor & Boksa, 1997). Post-pubertal rats delivered by C-section only however should display increases in DA levels in the PFC, nucleus accumbens, and striatum and consequently less DA transporter. Since this was not the

case, either DA transporter and/or TH is not affected by the insults of asphyxia and/or C-section delivery in post-pubertal rats in the nucleus accumbens, or there were errors in brain tissue analysis.

The antigen retrieval process required for the use of DA transporter compromised tissue stability and many samples were removed from severe damage during retrieval and immunohistochemical staining. A reduction in the number of slices and tissue damage in viable slices could have led to inaccuracies in average optical density measures of DA transporter in the nucleus accumbens. Furthermore, slices were investigated for DA transporter and TH cell counting (stereology), however the antibody did not penetrate the tissue enough for accurate and reliable cell counting. Therefore, DA transporter and TH levels in the nucleus accumbens and throughout the MLDA should be performed again in future studies with thicker slices to reduce brain tissue damage and more rigorous pilot studies must be performed to effectively identify DA transporter and TH within the entire slice measured.

Conclusion

Social behavioral and dopaminergic brain analyses in this study demonstrate that asphyxia and/or C-section delivery leads to overactive DA and potential decreased functioning of the HPA axis in early adulthood. Post-pubertal C-NON rats displayed longer bouts of anogenital sniffing, which can be explained by either increased release of DA in the caudate putamen, increased motivation to sniff from decreased D2 receptors in the caudate putamen, and/or deficits in odor discrimination from increased D2 activation in the olfactory bulbs. Post-pubertal C-APX rats displayed decreased latency to approach intruders, which lead to increased aggressive-offensive attacks from intruders and thus increased aggressive-defensive responses from C-APX rats. Increased aggressive-defensive responses suggest C-APX rats might have higher levels of stress hormone release during social interaction and potential deficits in HPA axis functioning.

However, decreased latency of approach behavior, which might have led to increased aggressive behavior could have resulted from increased "curiosity". Future studies must replicate our social behavioral findings and measure CORT levels prior to, during and after social interactions to determine differences in stress responses and deficits in HPA axis functioning in rats asphyxiated and/or delivered by C-section. Nonetheless, an elevated catecholamine surge during asphyxiation and/or an absence of a catecholamine surge at the time of birth results in over-active DA systems within the brain and increases in DA mediated behaviors associated with motivated behaviors such as anogenital sniffing and approaching.

REFERENCES

Albert, D. J., Wlash, M. L., Gorzalka, B. B., Mendelson, S., & Zalys, C. (1986). Intermale social aggression: suppression by medial preoptic area lesions. *Physiology and Behavior*, *38*, 169–173.

Almqvist, C., Cnattingius, S., Lichtenstein, P., & Lundholm, C. (2012). The impact of birth mode of delivery on childhood asthma and allergic diseases–a sibling study. *Clinical & Experimental Allergy*, *42*(9), 1369–1376. doi:10.1111/j.1365-2222.2012.04021.x

Barber, E. L., Lundsberg, L., Belanger, K., Pettker, C. M., Funai, E. F., & Iluzzi, J. L. (2011). Contributing Indications to the Rising Cesarean Delivery Rate. *Obstetrics and Gynecology*, *118*(1), 29–38. doi:10.1097/AOG.0b013e31821e5f65

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research. Brain Research Reviews*, *28*(3), 309–369.

Bjelke, B., Andersson, K., Ogren, S. O., & Bolme, P. (1991). Asphytic lesion: proliferation of tyrosine-hydroxylase-immunoreactive nerve cell bodies in the rat substantia nigra and functional changes in dopamine neurotransmission. *Brain Research*, *543*(1), 1–9.

Boksa, P. (1997). Early development profiles of corticosterone are altered by birth condition in the rat: A comparison of vaginal birth, cesarean section, and cesarean section with added anoxia. *Pediatric Research*, *41*(1), 34–43.

Boksa, P., & El-Khodor, B. F. (2003). Birth insult interacts with stress at adulthood to alter dopaminergic function in animal modles: possible implications for schizophrenia and other disorders. *Neuroscience and Biobehavioral Reviews*, *27*, 91–101

Boksa, P., Krishnamurthy, A., & Sharma, S. (1996). Hippocampal and hypothalamic type I corticosteroid receptor affinities are reduced in adult rats born by a caesarean procedure with or without an added period of anoxia. *Neuroendocrinology*, *64*(1), 25–34.

Boksa, P., & Zhang, Y. (2008). Epinephrine administration at birth prevents long-term changes in dopaminergic parameters caused by cesarean section birth in the rat. *Psychopharmacology*, *200*(3), 381–391.

Boksa, P., Zhang, Y., & Besawros, A. (2002). Dopamine D1 receptor changes due to caesarean section birth: effects of anesthesia, developmental time course, and functional consequences. *Experimental Neurology*, *175*, 388–397.

Brake, W., Sullivan, R., & Gratton, A. (2000). Perinatal distress leads to lateralized medial prefrontal cortical dopmaine hypofunction in adult rats. *Journal of Neuroscience*, *20*(14), 538–543.

Brenner, K., Liu, A., Laplante, D. P., Lupien, S., Pruessner, J. C., Ciampi, A., … King, S. (2009). Cortisol response to a psychosocial stressor in schizophrenia: blunted, delayed, or normal? *Psychoneuroendocrinology*, *34*, 859–868.

Burns, L. R., Geller, S. E., & Wholey, D. R. (1995). The Effect of Physician Factors on the Cesarean Section Decision. *Medical Care*, *33*(4), 365–382.

Chen, K., Holschneider, D. P., Wu, W., Rebrin, I., & Shih, J. C. (2004). A Spontaneous Point Mutation Produces Monoamine Oxidase A/B Knock-out Mice with Greatly Elevated Monoamines and Anxiety-like Behavior. *Journal of Biological Chemistry*, *279*(38), 39645–39652. doi:10.1074/jbc.M405550200

Chen, Y., Engidawork, E., Loidl, F., Elisabetta, D., Goiny, M., Lubec, G., … Herrera-Marschitz, M. (1997). Short- and long-term effects of perinatal asphyxia on monamine, amino acid

and glycolysis product levels measured in the basal ganglia of the rat. *Developmental Brain Research*, *104*(1), 19–30.

Clancy, B., Finlay, B. L., Darlington, R. B., & Anand, K. J. (2007). Extrapolating brain development from experimental species to humans. *Neurotoxicology*, *28*, 931–937.

Coppola, D. M., & Millar, L. C. (1994). Stimulus access to the accessory olfactory system in the prenatal and perinatal rat. *Neuroscience*, *60*(2), 463–468.

Costa, R., Lin, S. C., Cyr, M., Gainetdinov, R., & Caron, M. (2006). Rapid alterations in corticostriatal enesemble coordination during acute dopamine-dependent motor dysfunction. *Neuron*, *52*, 359–369.

Crawley, J. N., Gerfen, C. R., Rogawski, M. A., Sibley, D. R., Skolnick, P., & Wray, S. (2007). *Short protocols in neuroscience*. Hoboken, NJ: John Wiley & Sons.

De Boer, S. F., van der Vegt, B. J., & Koolhaas, J. M. (2003). Individual variation in aggression of feral rodent strains: a standard for the genetics of aggression and violence? *Behavior Genetics*, *33*(5), 485–501.

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Human Development*, *3*, 79–83.

Doty, R. L., Li, C., Bagla, R., Huang, W., Pfeiffer, C., Brosvic, G. M., & Risser, J. M. (1998). SKF 38393 enhances odor detection performance. *Psychopharmacology*, *136*(1), 75–82.

Doty, R. L., & Risser, J. M. (1989). Influence of the D-2 dopamine receptor agonist quinpirole on the odor detection performance of rats before and after spiperone administration. *Psychopharmacology*, *98*(3), 310–315.

Dreher, J., & Jackson, D. (1989). Role of D1 and D2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. *Brain Research*, *487*(2), 267–277.

Ecker, J. L., Chen, K. T., Cohen, A. P., Riley, L. E., & Lieberman, E. S. (2001). Increased risk of cesarean delivery with advancing maternal age: Indications and associated factors in nulliparous women. *American Journal of Obstetrics and Gynecology*, *185*(4), 883–887. doi:10.1067/mob.2001.117364

El-Khodor, B. F., & Boksa, P. (1997). Long-Term Reciprocal Changes in DA Levels in Prefrontal Cortex versus Nucleus Accumbens in Rats Born by Caesarean Section Compared to Vaginal Birth. *Experimental Neurology*, *145*, 118–129.

El-Khodor, B. F., & Boksa, P. (1998). Birth insult increases amphetamine-induced behavioral respones in the adult rat. *Neuroscience*, *87*(4), 893–904.

El-Khodor, B. F., & Boksa, P. (2001). Caesarean section birth produces long-term alterations in D1 receptors and in stress-induced regulation of D3 and D4 receptors in the rat. *Neuropsychopharmacology*, *25*(3), 423–439.

El-Khodor, B. F., & Boksa, P. (2002). Birth insult and stress interact to alter dopamine transporter binding in rat brain. *NeuroReport*, *13*(2), 1–6.

Escanilla, O., Yuhas, C., Marzan, D., & Linster, C. (2009). Dopaminergic modulation of olfactory bulb processing affects odor discrimination learning in rats. *Behavioral Neuroscience*, *123*(4), 828–833. doi:10.1037/a0015855

Fernandez-Espejo, E., & Mir, D. (1990). Ethological analysis of the male rat's socioagnostic behavior in a resident-intruder paradigm. *Aggressive Behavior*, *16*, 41–55.

File, S. E. (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *Journal of Neuroscience Methods*, *2*, 219–238.

Geddes, J., Verdoux, H., Takei, N., Lawrie, S., Bovet, P., & Eagles, J. (1999). Schizophrenia and complications of pregnancy and labor: An individual pateient data meta-analysis. *Schizophrenia Bulletin*, *25*, 413–423.

Giorgio, O., Pibiri, M. G., Loi, R., & Corda, M. G. (1993). Chronic treatment with SCH 23390 increases the production rate of dopamine D1 receptors in the nigro-striatal system of the rat. *European Journal of Pharmacology*, *245*, 139–145.

Graybiel, A. M., & Saka, E. (2004). The Basal Ganglia and the Control of Action. In *The Cognitive Neurosciences* (3rd ed., pp. 495–510). Cambridge, MA: The MIT Press.

Hagberg, B., Hagberg, G., Lewerth, A., & Lindberg, U. (1999). Mild mental retardation in Swedish school children. *Acta Paediatrica Scandinavica*, *70*, 445–452.

Halpern, M., & Martínez-Marcos, A. (2003). Structure and function of the vomeronasal system: an update. *Progress in Neurobiology*, *70*(3), 245–318. doi:10.1016/S0301-0082(03)00103-5

Hamilton, B., Martin, J., & Ventura, S. (2009). Births: Preliminary Data for 2007. *National Vital Statistics Reports*, *57*(12), 1–23.

Harris, L. W., Guest, P. C., Wayland, M. T., Umrania, Y., Krishnamurthy, D., Rahmoune, H., & Bahn, S. (2012). Schizophrenia: Metabolic aspects of aetiology, diagnosis and future treatment strategies. *Psychoneuroendocrinology*.

Henry, J. P., & Stephens, P. M. (1977). *Stress, health, and the social environment: A sociobiological approach to medicine* (1st ed.). New York, NY: Springer.

Hoeger, H., Engelmann, M., Bernert, G., Seidl, R., Bubna-Littitz, H., Mosgoeller, W., … Lubec, G. (2000). Long term neurological and behavioral effects of graded perinatal asphyixa in the rat. *Life Sciences*, *66*, 947–962.

Irestedt, L., Lagercrantz, H., Hjemdahl, P., Hägnevik, K., & Belfrage, P. (1982). Fetal and maternal plasma catecholamine levels at elective cesarean section under general or epidural anesthesia versus vaginal delivery. *American Journal of Obstetrics and Gynecology*, *142*(8), 1004–1010.

Jones, C. T. (1980). Circulating catecholamines in the fetus, their origin, actions and significance. In *Biogenic amines in development* (pp. 63–68). Amsterdam: Elsevier/North Holland Biomedical Press.

Joyce, J. N. (1990). Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine: II. Effects of 6-hydroxydopamine or colchicine microinjections into the VTA or reserpine treatment. *Experimental Neurology*, *113*, 277–290.

Juarez, I., Silva-Gomez, A. B., Peralta, F., & Flores, G. (2003). Anoxia at birth induced hyperresponsiveness to amphetamine and stress in postpubertal rats. *Brain Research*, *992*, 281–287.

Kandel, E., Schwartz, J., & Jessel, T. (2000). *Principles of Neural Science* (4th ed.). New York, NY: McGraw-Hill Companies Inc.

Kienast, T., & Heinz, A. (2006). Dopamine in the diseased brain. *CNS & Neurological Disorders - Drug Targets*, *5*, 109–131.

Koolhaas, J., Schuurman, T., & Wiepkema, P. (1980). The organization of intraspecific agnostic behaviour in the rat. *Progress in Neurobiology*, *15*, 247–298.

Lagercrantz, H., & Bistoletti, P. (1973). Catecholamine release in the newborn infant at birth. *Pediatric Research*, *11*, 889–893.

Lagercrantz, H., & Slotkin, T. (1986). The "stress" of being born. *Scientific American*, *254*(4), 100–107.

LaHoste, G. J., & Marshall, J. F. (1991). Chronic eticlopride and dopamine denervation induce equal nonadditive increases in striatal D2 receptor density: Autoradiographic evidence against the dual mechanism hypothesis. *Neuroscience*, *41*, 473–481.

Langen, M., Kas, M. J. H., Staal, W. G., van Engeland, H., & Durston, S. (2011). The neurobiology of repetitive behavior: of mice…. *Neuroscience and Biobehavioral Reviews*, *35*(3), 345–355. doi:10.1016/j.neubiorev.2010.02.004

Lanuza, E., Novejarque, A., Martínez-Ricós, J., Martínez-Hernández, J., Agustín-Pavón, C., & Martínez-García, F. (2008). Sexual pheromones and the evolution of the reward system of the brain: the chemosensory function of the amygdala. *Brain Research Bulletin*, *75*(2-4), 460–466. doi:10.1016/j.brainresbull.2007.10.042

Laplante, F., Brake, W., Chehab, S., & Sullivan, R. (2012). Sex differences in the effects of perinatal anoxia on dopamine function in rats. *Neuroscience Letters*, *506*(1), 89–93.

Malamud, N. (1981). Status marmoratus: a form of cerebral palsy following either birth injury or inflammation of the central nervous system. *Journal of Pediatrics*, *37*, 610–619.

Martin, J. A., Hamilton, B. E., Ventura, S. J., Osterman, M. J. K., Wilson, E. C., & Matthews, T. J. (2012). Births: Final data for 2010. *National Vital Statistics Reports*, *61*(1), 1–71.

McIntosh, D. E., Mulkins, R. S., & Dean, R. S. (1995). Utilization of maternal perinatal risk indicators in the differential diagnosis of ADHD and UADD children. *International Journal of Neuroscience*, *81*(1-2), 35–46.

McNeil, T., Cantor-Graae, E., & Ismail, B. (2000). Obstetric complications and cogenital malformation in schizophrenia. *Brain Research Reviews*, *31*, 166–178.

Michel, G. F. (2010). Behavioral science, engineering, and poetry revisited. *Journal of Comparative Psychology (Washington, D.C.: 1983)*, *124*(3), 336–341. doi:10.1037/a0017355

Michel, G. F., & Moore, C. L. (1995). Animal Behavior, Ethology, and Human Development. In *Developmental Psychobiology: An Interdisciplinary Science* (pp. 383–428). Cambridge, MA: The MIT Press.

Misslin, R., Herzog, F., Koch, B., & Ropartz, P. (1982). Effects of isolation, handling, and novelty on the pituitary-adrenal response in the mouse. *Psychoneuroendocrinology*, *7*(2-3), 217–221.

Morgan, J. J., Kleven, G. A., Tulbert, C. D., Olson, J., & Ronca, A. E. (2012). Outcomes of intrauterine asphyxia in the full term rat (Vol. Online Abstract, p. No. 861.13/K6). Presented at the Society for Neuroscience, New Orleans, LA.

Moyer, K. (1971). A preliminary physiologicial model of aggressive behavior. In *The Physiology of Aggression and Defeat* (pp. 223–263). New York, NY: Plenum Press.

Murrin, L. C., & Zeng, W. (1990). Ontogenty of dopamine D1 receptors in rat forebrain: A quantitative autoradiographic study. *Developmental Brain Research*, *57*, 7–13.

Nath, S., Roy, R., & Mukherjee, S. (2012). Perinatal complications associated with autism--a case control study in a neurodevelopment and early intervention clinic. *Journal of the Indian Medical Association*, *110*(8), 526–529.

NICE. (2007). CG55 Intrapartum care: NICE guideline. Retrieved from http://publications.nice.org.uk/intrapartum-care-cg55/introduction

Palsdottir, K., Daqbjartsson, A., Throkelsson, T., & Hardardottir, H. (2007). Birth asphyxia and hypoxic ischemic encephalopathy, incidence and obstetric risk factors. *Laeknabladid*, *93*(9), 595–601.

Panksepp, J., Knutson, B., & Burgdorf, J. (2002). The role of brain emotional systems in addictions: a neuro-evolutionary perspective and new “self-report” animal model. *Addiction (Abingdon, England)*, *97*(4), 459–469.

Pletzer, B., Klimesch, W., Oberascher-Holzinger, K., & Kerschbaum, H. H. (2007). Corticosterone response in a resident-intruder-paradigm depends on social state and

coping style in adolescent male Balb-C mice. *Neuro Endocrinology Letters*, *28*(5), 585–590.

Rice, J. E., 3rd, Vannucci, R. C., & Brierley, J. B. (1981). The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Annals of Neurology*, *9*(2), 131–141. doi:10.1002/ana.410090206

Ronca, A. E., Abel, R. A., Ronan, P. J., Renner, K. J., & Alberts, J. R. (2006). Effects of labor contractions on catecholamine release and breathing frequency in newborn rats. *Behavioral Neuroscience*, *120*(6), 1308–1314.

Ronca, A. E., & Alberts, J. R. (1995). Simulated uterine contractions facilitate fetal and newborn respiratory behavior in rats. *Physiology & Behavior*, *58*(5), 1035–1041. doi:10.1016/0031-9384(95)00155-C

Siegel, S. (1956). *Nonparametric statistics for the behavioral sciences*. New York, NY: McGraw-Hill Companies Inc.

Spruijt, B. M., van Hoof, J. A., & Gispen, W. H. (1992). Ethology and neurobiology of grooming behavior. *Physiology Review*, *72*(3), 825–852.

Ungethum, U., Chen, Y., Gross, J., Bjelke, B., Bolme, P., & Eneroth, P. (1996). Effects of perinatal asphyxia on teh mesostriatial/mesolimbic dopamine system of neonatal and 4-week-old male rats. *Experimental Brain Research*, *112*, 403–410.

VanderWeele, T. J., Lantos, J. D., & Lauderdale, D. S. (2012). Rising preterm birth rates, 1989–2004: Changing demographics or changing obstetric practice? *Social Science & Medicine*, *74*(2), 196–201. doi:10.1016/j.socscimed.2011.10.031

Venerosi, A., Cutuli, D., Chiarotti, F., & Calamandrei, G. (2006). C-section birth per se or followed by acute global asphyxia altered emotional behaviorin neonate and adult rats. *Behavioral Brain Research*, *168*, 56–63.

Venerosi, A., Valanzano, A., Cirulli, F., & Calamandrei, G. (2004). Acute global anoxia during C-section birth affects dopamine-mediated behavioural responses and reactivity to stress. *Behavioral Brain Research*, *154*, 155–164.

Vezina, P., Blanc, G., Glowinski, J., & Tassin, J.-P. (1991). Opposed Behavioural Outputs of Increased Dopamine Transmission in Prefrontocortical and Subcortical Areas: A Role for the Cortical D-1 Dopamine Receptor. *The European Journal of Neuroscience*, *3*(10), 1001–1007.

Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Progress in Neurobiology*, *65*(5), 427–451.

Weitzdoerfer, R., Pollack, A., & Lubec, B. (2004). Perinatal asphyxia in the rat has lifelong effects on morphology. *Seminars in Perinatology*, *28*(4), 249–256.

Welberg, L., & Seckl, J. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology*, *13*(2), 113–128.

Wilkonson, P. O., & Goodyer, I. M. (2011). Childhood adversity and allostatic overload of the hypothalamic-pituitary-adrenal axis: A vulnerability model for depressive disorders. *Development and Psychopathology*, *23*, 1017–1037.

Zain, H. A., Wright, J. W., Parrish, G. E., & Diehl, S. J. (1998). Interpreting the fetal heart rate tracing. Effect of knowledge of neonatal outcome. *The Journal of Reproductive Medicine*, *43*(4), 367–370.

Zhang, J., Troendle, J., Reddy, U. M., Laughon, S. K., Branch, D. W., Burkman, R., … Van Veldhuisen, P. (2010). Contemporary Cesarean Delivery Practice in the United States. *American Journal of Obstetrics and Gynecology*, *203*(4), 326.e1–326.e10. doi:10.1016/j.ajog.2010.06.058

APPENDIX A

TABLES AND FIGURES

Table 1. Recorded Social Behaviors

	Behavior	Operational Definition
Social Investigatory Behavior	Approach (L)	Approaching rat walks or moves head towards the non-locomoting partner rat
	Sniffing (L)	Sniffing of the partner's body, including head
	Anogential Sniffing	Sniffing of the partner's anogential area
	Follow/Chase	Following rat locomotes towards locomoting partner rat
	Mount Rear	Front Paws on back of partner, no locomotion
	Mount Front	Front paws on head of partner, no locomotion
	Crawl Over	Rat crawls over the other
	Allogrooming (L)	Dominant rat mounts then nibbling of the fur of the partner
Non-social Investigatory Behavior	Exploring Rearing	Sniffing and walking around arena Placing front paws on arena wall, raising body vertically
	Self-Grooming	Face washing, licking, nibbling and wiping the fur of self
Aggressive Defensive Behavior	Freezing/Sitting	Rat is immobile in response to partner
	Withdrawal	Rat forces a crawl over or quick trot to escape partner
Aggressive Offensive Behavior	Nape Attack (L)	One attacks nape (very short), leading the other to respond with an attack or withdrawal
	Lateral Attack (Threat)	Attacking rat pushes nose into side of partner rat, lifting it in the air

Table 2. Frequency and Duration of Investigative & Aggressive Behavior

Grouped Social Investigative Behavior		
	Frequency	Duration
C-APX x C-NON	W = 10, p>.05	W = 13, p>.05
C-APX x VG	U = 19, p>.05	U = 12.5, p>.05
C-NON x VG	U = 26, p>.05	U = 33, p>.05

Grouped Non-Social Investigative Behavior		
	Frequency	Duration
C-APX x C-NON	W = 12, p>.05	W = 12, p>.05
C-APX x VG	U = 19.5, p>.05	U = 23, p>.05
C-NON x VG	U = 30, p>.05	U = 27, p>.05

Grouped Aggressive-Offensive Behavior		
	Frequency	Duration
C-APX x C-NON	W = 8.5, p>.05	W = 9, p>.05
C-APX x VG	U = 15, p>.05	U = 22.5, p>.05
C-NON x VG	U = 21, p>.05	U = 27, p>.05

Grouped Aggressive-Defensive Behavior		
	Frequency	Duration
C-APX x C-NON	W = 9 p>.05	W = 12, p>.05
C-APX x VG	U = 8.5, p<.01**	U = 10, p>.05
C-NON x VG	U = 30.5, p>.05	U = 32, p>.05

Figure 1. Median Duration per Anogential Sniff

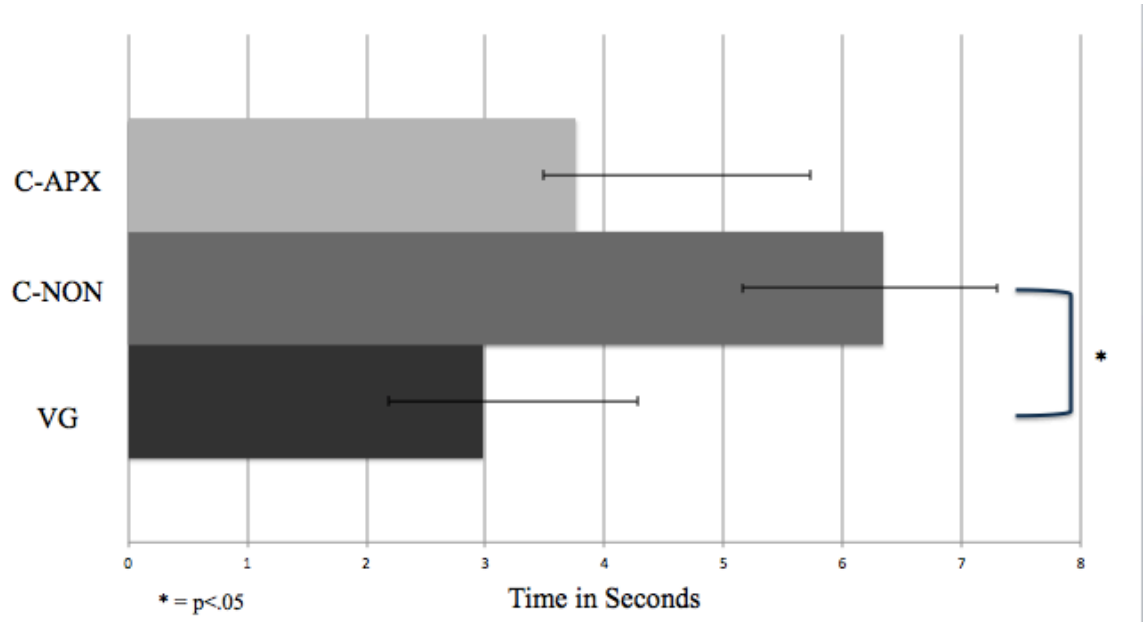

Figure 2. Median Frequency of Defensive Behavior

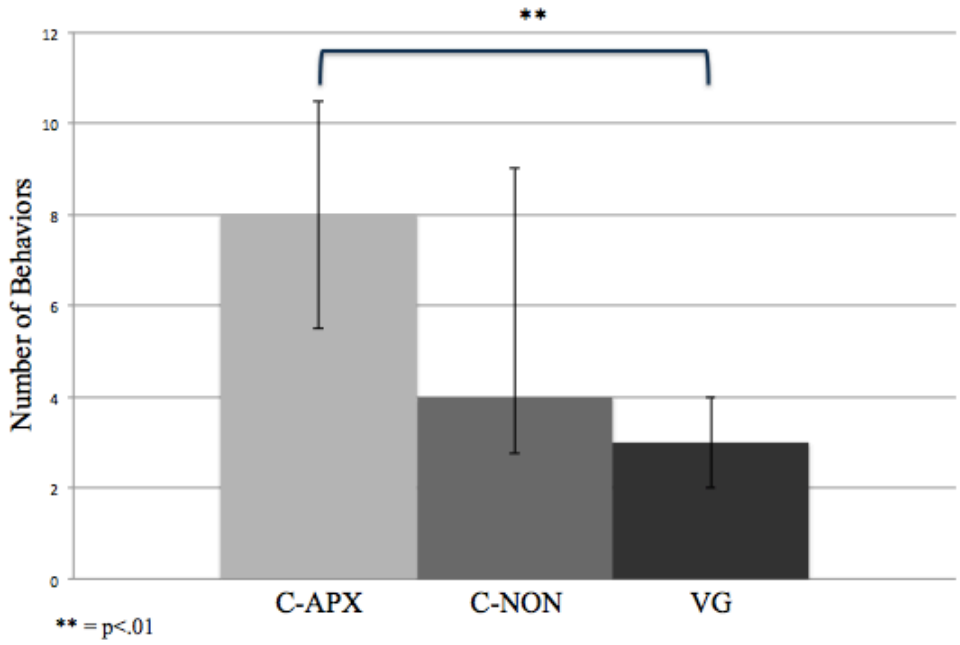

Figure 3. Median Frequency of Withdrawal Behavior

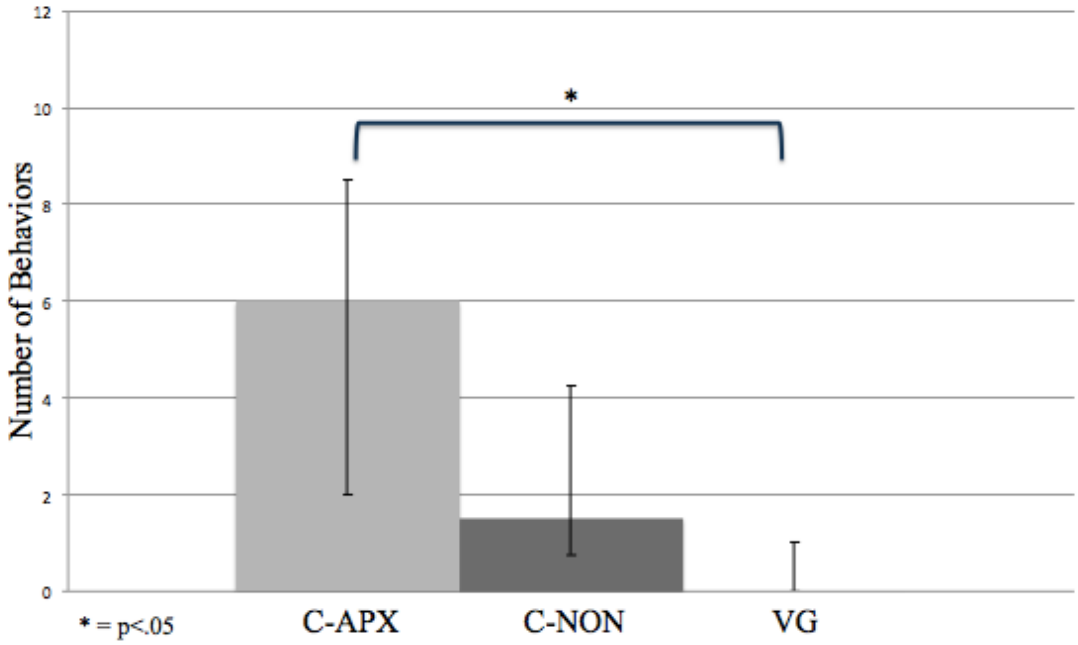

Figure 4. Median Frequency of Intruder Aggressive Behavior

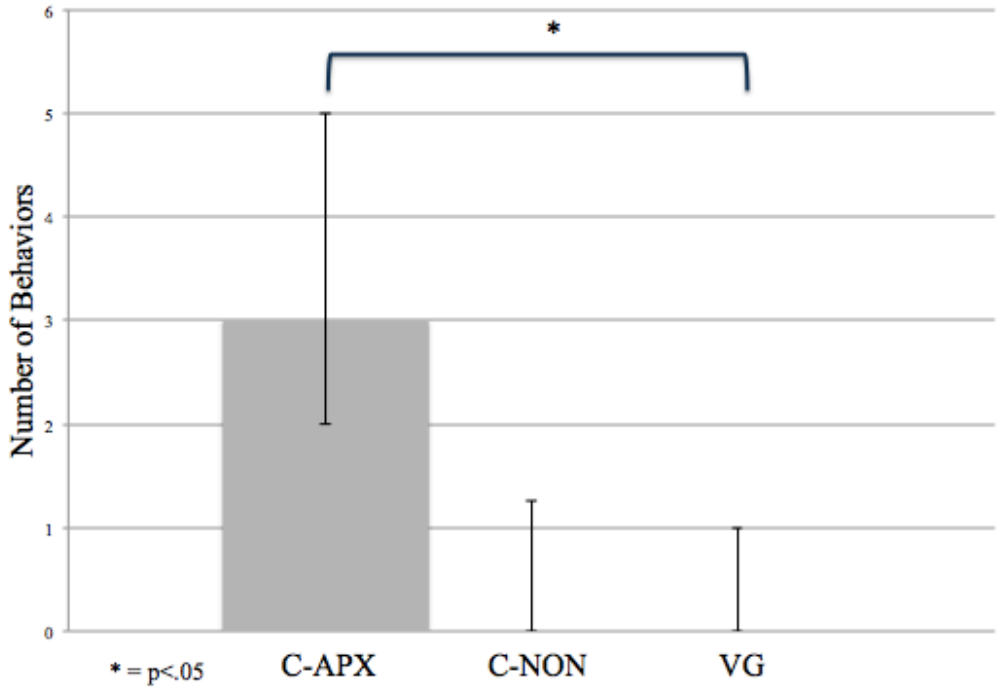

Figure 5. Resident Latency to Approach

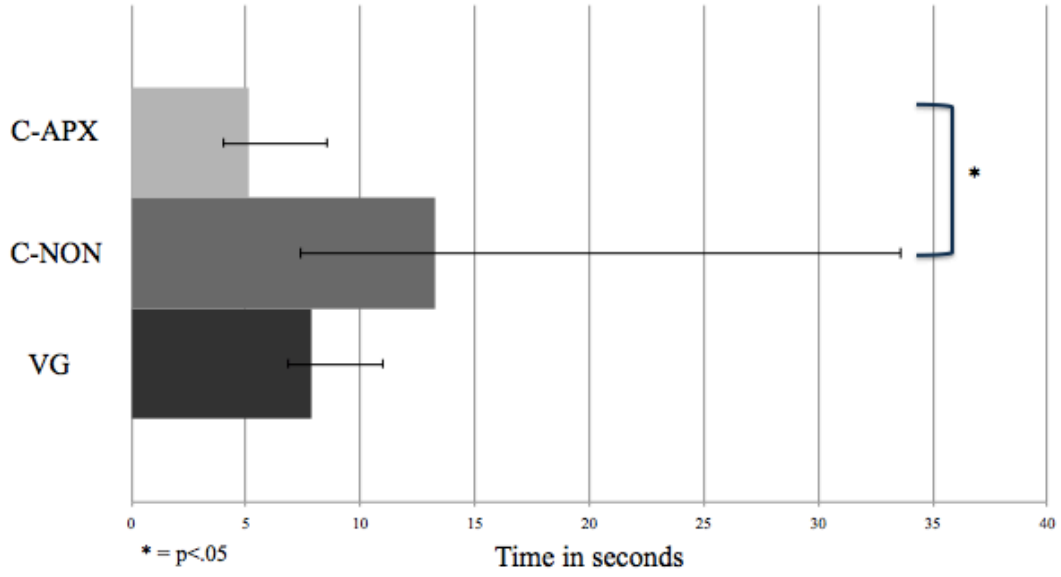

Figure 6. 1st Interaction

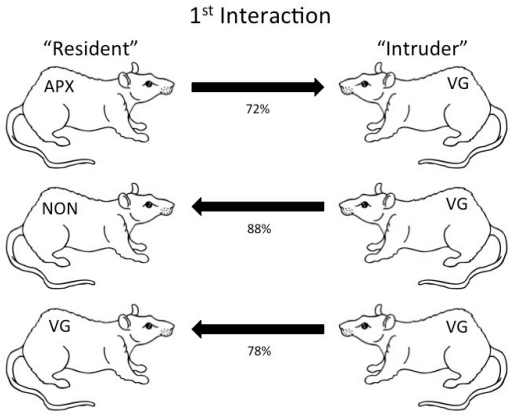

Figure 7. Median Frequency of Self-grooming Behavior

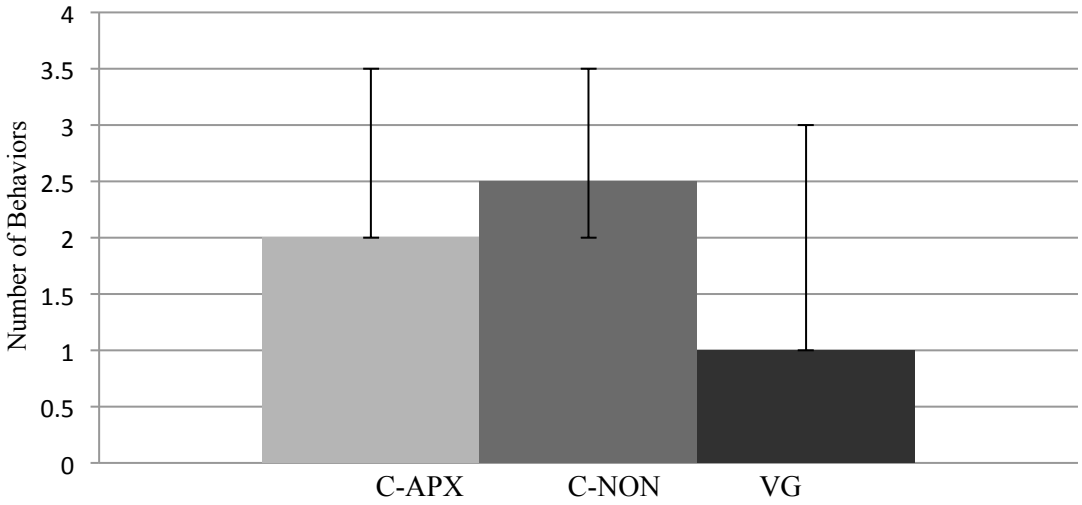

Table 3. Average Optical Density of DA Transporter and TH in the AccC, and AccS

DAT - Nucleus accumbens shell	
	AOD
C-APX x C-NON	W = 7, p>.05
C-APX x VG	U = 19, p>.05
C-NON x VG	U = 14, p>.05

DAT - Nucleus accumbens core	
	AOD
C-APX x C-NON	W = 5, p>.05
C-APX x VG	U = 20, p>.05
C-NON x VG	U = 17, p>.05

TH - Nucleus accumbens shell	
	AOD
C-APX x C-NON	W = 6, p>.05
C-APX x VG	U = 24, p>.05
C-NON x VG	U =15, p>.05

TH - Nucleus accumbens core	
	AOD
C-APX x C-NON	W = 7 p>.05
C-APX x VG	U = 15, p>.05
C-NON x VG	U = 13, p>.05